COMPOUNDS, COMPOSITIONS, AND METHODS

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application number 60/466,973, filed April 30, 2003 and of U.S. Provisional Patent Application number 60/467,165, filed April 30, 2003, each of which is incorporated herein by reference for all purposes.

FIELD OF THE INVENTION

[0002] This invention relates to compounds which are inhibitors of the fungal kinesin Kip1 and are useful in the treatment of fungal disorders.

BACKGROUND OF THE INVENTION

[0003] Pathogenic fungi occur worldwide and are major agricultural and health pests. Fungal infections in humans range from superficial and cutaneous to deeply invasive and disseminated.

[0004] In the past 20 years, the incidence of fungal infections has increased dramatically--along with the numbers of potentially invasive species. Indeed, fungal infections, once dismissed as a nuisance, have begun to spread so widely that they are becoming a major concern in hospitals and health departments. Fungal infections occur more frequently in people whose immune system is compromised or suppressed (e.g., because of organ transplantation, cancer chemotherapy, or the human immunodeficiency virus), who have been treated with broad-spectrum antibacterial agents, or who have been subject to invasive procedures (catheters and prosthetic devices, for example).

[0005] The 1980s and 1990s witnessed a steep rise in Candida and Aspergillus infections (Musial et al. (1988) Clin. Microb. Rev. 1(4):349-364; Saral (1991) Reviews of Infectious Dis. 13:487-492). Similar rises in zygomycosis, cryptococcosis, histoplasmosis and fusaria infection have also been noted.

[0006] There is intense interest in identifying new drugs with different modes of action against fungal infections. The current repertoire of antifungals has limitations

such as insufficient efficacy, the need for intravenous administration, serious side effects, and/or the appearance of resistant fungal strains.

[0007] Fungal kinesins, and more particularly the fungal kinesin Kip1 from Candida albicans, represent important new targets for antifungal drugs. Significantly, although fungi, like mammalian cells, are eukaryotes, there is relatively low homology between the fungal kinesin Kip1 and human Kip1. See, e.g., PCT Application No. PCT/US03/35669, which is incorporated herein by reference. As such, it is possible to specifically inhibit the fungal protein and not the human Kip1, thus, decreasing or even eliminating toxic side effects.

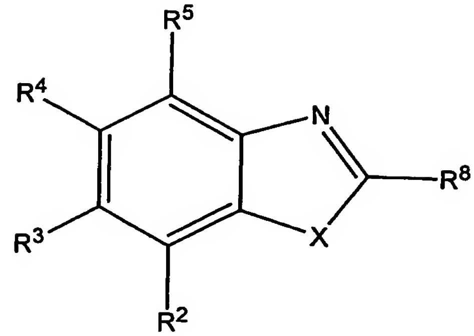
[0008] Currently, there is a paucity of agents that are effective in the treatment of fungal infections. There is a great need for agents that exploit new mechanisms of action and may have better outcomes in terms of relief of symptoms, safety, and patient mortality, both short-term and long-term. The present invention provides such agents and methods for their use.

SUMMARY

[0009] In accordance with the objects outlined above, the present invention provides compositions and methods that can be used to treat fungal infections. The compositions inhibit the fungal kinesin Kip1.

[0010] In one aspect, the invention relates to methods for the treatment of a fungal infection and more particularly, a fungal infection caused by a *Candida* species. such as *Candida albicans*, *Candida tropicalis*, *Candida (Torulopsis) glabrata*, *Candida parapsilosis*, *Candida lusitaneae*, *Candida rugosa,* and *Candida pseudotropicalis*. Fungal infections which can be inhibited or treated with compositions provided herein include candidiasis including but not limited to onchomycosis, chronic mucocutaneous candidiasis, oral candidiasis, epiglottistis, esophagitis, gastrointestinal infections, and genitourinary infections.

[0011]. In one aspect, the invention relates to compounds of Formula I



Formula I

wherein

X is $-NR^1-$, $-CH=N-$, $-N=CH-$, -CH=CH-, S, -(SO)-, -(SO_2)-, or O;

R^1 is hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, or optionally substituted heteroaralkyl;

R^2, R^3, R^4, and R^5 are independently hydrogen, optionally substituted acyl, optionally substituted alkyl, optionally substituted aralkyl, optionally substituted heteroaralkyl, optionally substituted alkoxy, optionally substituted aralkoxy, optionally substituted heteroaralkoxy, halogen, hydroxyl, nitro, cyano, optionally substituted amino, sulfonyl, sulfanyl, carboxy, optionally substituted alkoxycarbonyl, optionally substituted aminocarbonyl, optionally substituted aryl or optionally substituted heteroaryl; or R^3 and R^4, together with the carbons to which they are attached, form an optionally substituted 5- or 6-membered alicyclic ring; and

R^8 is hydrogen, cyano, halogen, optionally substituted acyl, optionally substituted lower alkyl, optionally substituted aryl, optionally substituted heteroaryl, sulfonyl, or optionally substituted amino;

provided that

R^4 is not trifluoromethyl when X is NR^1; R^5, R^3, R^2, and R^1 are hydrogen; and R^8 is optionally substituted lower alkyl;

R^3 is not trifluoromethoxy, optionally substituted lower alkyl or halo when X is S; R^5, R^4, and R^2 are hydrogen; and R^8 is amino;

X is not S, when R^3 and R^4, together with the carbons to which they are attached form a cyclohexyl ring; R^8 is amino; and R^5 and R^2 are hydrogen; and

R^4 is not lower alkyl or halo, when X is NR^1; R^5, R^3, R^2, and R^1 are hydrogen; and R^8 is amino;

including single stereoisomers, mixtures of stereoisomers, and pharmaceutically acceptable salts thereof. The compounds of Formula I are useful as active agents in practice of the methods of treatment and in manufacture of the pharmaceutical formulations of the invention, and as intermediates in the synthesis of such active agents.

[0012] In other aspects, the invention relates to a pharmaceutical formulation including a pharmaceutically acceptable excipient, and to a method of treatment for fungal infection, each entailing a therapeutically effective amount of a compound represented by Formula I.

[0013] Other aspects and embodiments will be apparent to those skilled in the art from the following detailed description.

DETAILED DESCRIPTION OF EXAMPLE EMBODIMENTS

Definitions

[0014] As used in the present specification, the following words and phrases are generally intended to have the meanings as set forth below, except to the extent that the context in which they are used indicates otherwise. The following abbreviations and terms have the indicated meanings throughout:

ATP	=	Adenosine triphosphate
c-	=	cyclo
BSA	=	Bovine Serum Albumin
dppf	=	(diphenylphosphino)ferrocene
DTT	=	dithiothreitol
EGTA	=	ethylene glycol-bis(b-aminoethyl ether) N,N,N',N'-tetraacetic acid free acid
eq.	=	equivalent
EtOAc	=	ethyl acetate
EtOH	=	ethanol
g	=	grams
h	=	hour
Me	=	methyl
min	=	minute

mL	=	milliliter
mmol	=	millimole
nM	=	nanomolar
nm	=	nanometer
Ph	=	phenyl
rt or RT	=	room temperature
s-	=	secondary
t-	=	tertiary
THF	=	tetrahydrofuran

[0015] As used herein, "a" or "an" means one or more. As used herein in the claim(s), when used in conjunction with the word "comprising", the words "a" or "an" means one or more than one. As used herein "another" means at least a second or more.

[0016] **Alkyl** is intended to include linear, branched, or cyclic hydrocarbon structures and combinations thereof. **Lower alkyl** refers to alkyl groups of from 1 to 5 carbon atoms. Examples of lower alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, s- and t-butyl and the like. Preferred alkyl groups are those of C_{20} or below. More preferred alkyl groups are those of C_{13} or below. Yet more preferred are alkyl groups of C_6 and below. Cycloalkyl is a subset of alkyl and includes cyclic hydrocarbon groups of from 3 to 13 carbon atoms. Examples of cycloalkyl groups include c-propyl, c-butyl, c-pentyl, norbornyl, adamantyl and the like. In this application, alkyl refers to alkanyl, alkenyl and alkynyl residues; it is intended to include cyclohexylmethyl, vinyl, allyl, isoprenyl and the like. Alkylene is another subset of alkyl, referring to the same residues as alkyl, but having two points of attachment. Examples of alkylene include ethylene ($-CH_2CH_2-$), propylene ($-CH_2CH_2CH_2-$), dimethylpropylene ($-CH_2C(CH_3)_2CH_2-$) and cyclohexylpropylene ($-CH_2CH_2CH(C_6H_{13})-$). When an alkyl residue having a specific number of carbons is named, all geometric isomers having that number of carbons are intended to be encompassed; thus, for example, "butyl" is meant to include n-butyl, sec-butyl, isobutyl and t-butyl; "propyl" includes n-propyl and isopropyl.

[0017] **Alkoxy** or **alkoxyl** refers to the group -O-alkyl, preferably including from 1 to 8 carbon atoms of a straight, branched, cyclic configuration and combinations thereof attached to the parent structure through an oxygen. Examples include methoxy,

ethoxy, propoxy, isopropoxy, cyclopropyloxy, cyclohexyloxy and the like. Lower alkoxy refers to groups containing one to four carbons.

[0018] **Acyl** refers to groups of from 1 to 8 carbon atoms of a straight, branched, cyclic configuration, saturated, unsaturated, aromatic and combinations thereof, attached to the parent structure through a carbonyl functionality. One or more carbons in the acyl residue can be replaced by nitrogen, oxygen or sulfur as long as the point of attachment to the parent remains at the carbonyl. Examples include acetyl, benzoyl, propionyl, isobutyryl, t-butoxycarbonyl, benzyloxycarbonyl and the like. Lower-acyl refers to groups containing one to four carbons.

[0019] **Alicyclic** refers to an aliphatic, cyclic residue. Examples of alicyclic groups include cyclopentyl, cyclohexyl, and the like.

[0020] **Amino** refers to the group $-NH_2$. **Substituted amino** refers to the group -NHR or -NRR where each R is independently chosen from the group: optionally substituted alkyl-, optionally substituted alkoxy, optionally substituted aminocarbonyl-, optionally substituted aryl-, optionally substituted heteroaryl-, optionally substituted heterocyclyl-, acyl-, alkoxycarbonyl-, sulfanyl-, sulfinyl and sulfonyl-, e.g., diethylamino, methylsulfonylamino, furanyl-oxy-sulfonamino. Substituted amino includes the groups $-NR^cCOR^b$, $-NR^cCO_2R^a$, and $-NR^cCONR^bR^c$, where

R^a is an optionally substituted C_1-C_6 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, or heteroaryl-C_1-C_4 alkyl- group;

R^b is H or optionally substituted C_1-C_6 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, or heteroaryl-C_1-C_4 alkyl- group; and

R^c is hydrogen or C_1-C_4 alkyl-; and where each optionally substituted R^b group is independently unsubstituted or substituted with one or more substituents independently chosen from C_1-C_4 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, heteroaryl-C_1-C_4 alkyl-, C_1-C_4 haloalkyl-, $-OC_1$-C_4 alkyl, $-OC_1$-C_4 alkylphenyl, $-C_1$-C_4 alkyl-OH, $-OC_1$-C_4 haloalkyl, halogen, -OH, $-NH_2$, $-C_1$-C_4 alkyl-NH_2, $-N(C_1$-C_4 alkyl)(C_1-C_4 alkyl), $-NH(C_1$-C_4 alkyl), $-N(C_1$-C_4 alkyl)(C_1-C_4 alkylphenyl), $-NH(C_1$-C_4 alkylphenyl), cyano, nitro, oxo (as a substitutent for heteroaryl), $-CO_2H$, $-C(O)OC_1$-C_4 alkyl, $-CON(C_1$-C_4 alkyl)(C_1-C_4 alkyl), $-CONH(C_1$-C_4 alkyl), $-CONH_2$, $-NHC(O)(C_1$-C_4 alkyl), -NHC(O)(phenyl), $-N(C_1$-C_4 alkyl)C(O)(C_1-C_4 alkyl), $-N(C_1$-C_4 alkyl)C(O)(phenyl), $-C(O)C_1$-C_4 alkyl, $-C(O)C_1$-C_4 phenyl, $-C(O)C_1$-C_4 haloalkyl, $-OC(O)C_1$-C_4 alkyl, $-SO_2(C_1$-C_4 alkyl), $-SO_2$(phenyl), $-SO_2(C_1$-C_4 haloalkyl), $-SO_2NH_2$, $-SO_2NH(C_1$-C_4 alkyl),

-SO_2NH(phenyl), -$NHSO_2$(C_1-C_4 alkyl), -$NHSO_2$(phenyl), and -$NHSO_2$(C_1-C_4 haloalkyl).

[0021] **Aminocarbonyl** refers to the group -$CONR^bR^c$, where

R^b is H or optionally substituted C_1-C_6 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, or heteroaryl-C_1-C_4 alkyl- group; and

R^c is hydrogen or C_1-C_4 alkyl-; and

where each optionally substituted R^b group is independently unsubstituted or substituted with one or more substituents independently chosen from C_1-C_4 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, heteroaryl-C_1-C_4 alkyl-, C_1-C_4 haloalkyl-, -OC_1-C_4 alkyl-, -OC_1-C_4 alkylphenyl, -C_1-C_4 alkyl-OH, -OC_1-C_4 haloalkyl, halogen, -OH, -NH_2, -C_1-C_4 alkyl-NH_2, -N(C_1-C_4 alkyl)(C_1-C_4 alkyl), -NH(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)(C_1-C_4 alkylphenyl), -NH(C_1-C_4 alkylphenyl), cyano, nitro, oxo (as a substitutent for heteroaryl), -CO_2H, -C(O)OC_1-C_4 alkyl, -CON(C_1-C_4 alkyl)(C_1-C_4 alkyl), -CONH(C_1-C_4 alkyl), -$CONH_2$, -NHC(O)(C_1-C_4 alkyl), -NHC(O)(phenyl), -N(C_1-C_4 alkyl)C(O)(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)C(O)(phenyl), -C(O)C_1-C_4 alkyl, -C(O)C_1-C_4 phenyl, -C(O)C_1-C_4 haloalkyl, -OC(O)C_1-C_4 alkyl, -SO_2(C_1-C_4 alkyl), -SO_2(phenyl), -SO_2(C_1-C_4 haloalkyl), -SO_2NH_2, -SO_2NH(C_1-C_4 alkyl), -SO_2NH(phenyl), -$NHSO_2$(C_1-C_4 alkyl), -$NHSO_2$(phenyl), and -$NHSO_2$(C_1-C_4 haloalkyl). Aminocarbonyl is meant to include carbamoyl-; lower-alkyl carbamoyl-; benzylcarbamoyl-; phenylcarbamoyl-; methoxymethyl-carbamoyl-; and the like.

[0022] **Aralkyl** refers to a residue in which an aryl moiety is attached to the parent structure via an alkyl residue. Examples include benzyl, phenethyl, phenylvinyl, phenylallyl and the like. **Heteroaralkyl** refers to a residue in which a heteroaryl moiety is attached to the parent structure via an alkyl residue. Examples include furanylmethyl, pyridinylmethyl, pyrimidinylethyl and the like.

[0023] **Aralkoxy** refers to the group –O–aralkyl. Similarly, **heteroaralkoxy** refers to the group –O–heteroaralkyl; **aryloxy** refers to the group –O–aryl; and **acyloxy** refers to the group –O—acyl.

[0024] **Aryl** and **heteroaryl** refer to a 5- or 6-membered aromatic or heteroaromatic ring containing 0 to 4 heteroatoms selected from O, N, and S; a bicyclic 9- or 10-membered aromatic or heteroaromatic ring system containing 0 to 4 (or more) heteroatoms selected from O, N, and S; or a tricyclic 12- to 14-membered aromatic or heteroaromatic ring system containing 0 to 4 (or more) heteroatoms selected from O, N,

and S. The aromatic 6- to 14-membered carbocyclic rings include, e.g., benzene, naphthalene, indane, tetralin, and fluorene and the 5- to 10-membered aromatic heterocyclic rings include, e.g., imidazole, pyridine, indole, thiophene, benzopyranone, thiazole, furan, benzimidazole, quinoline, isoquinoline, quinoxaline, pyrimidine, pyrazine, tetrazole and pyrazole.

[0025] **Aryloxy** and **heteroaryloxy** refer to aryl and heteroaryl groups, respectively, attached to the parent structure through an oxygen.

[0026] **ATPase** refers to an enzyme that hydrolyzes ATP. ATPases include proteins comprising molecular motors such as the myosins.

[0027] **Halogen** or **halo** refers to fluorine, chlorine, bromine or iodine. Fluorine, chlorine and bromine are preferred. Dihaloaryl, dihaloalkyl, trihaloaryl etc. refer to aryl and alkyl substituted with a plurality of halogens, but not necessarily a plurality of the same halogen; thus 4-chloro-3-fluorophenyl is within the scope of dihaloaryl.

[0028] **Heterocyclyl** means a cycloalkyl or aryl residue in which one to four of the carbons is replaced by a heteroatom such as oxygen, nitrogen or sulfur. Examples of heterocycles that fall within the scope of the invention include imidazoline, pyrrolidine, pyrazole, pyrrole, indole, quinoline, isoquinoline, tetrahydroisoquinoline, benzofuran, benzodioxan, benzodioxole (commonly referred to as methylenedioxyphenyl, when occurring as a substituent), tetrazole, morpholine, thiazole, pyridine, pyridazine, piperidine, pyrimidine, thiophene, furan, oxazole, oxazoline, isoxazole, dioxane, tetrahydrofuran and the like. **N-Heterocyclyl** refers to a nitrogen-containing heterocycle as a substituent residue. The term heterocyclyl encompasses heteroaryl, which is a subset of heterocyclyl. Examples of N-heterocyclyl residues include 4-morpholinyl, 4-thiomorpholinyl, 1-piperidinyl, 1-pyrrolidinyl, 3-thiazolidinyl, piperazinyl and 4-(3,4-dihydrobenzoxazinyl). Examples of substituted heterocyclyl include 4-methyl-1-piperazinyl and 4-benzyl-1-piperidinyl.

[0029] **Isolated, purified,** or **biologically pure** refer to material that is substantially or essentially free from components that normally accompany it as found in its native state. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography.

[0030] "**Isomers**" are different compounds that have the same molecular formula. "**Stereoisomers**" are isomers that differ only in the way the atoms are arranged in space.

"**Enantiomers**" are a pair of stereoisomers that are non-superimposable mirror images of each other. A 1:1 mixture of a pair of enantiomers is a "racemic" mixture. The term "(.±.)" is used to designate a racemic mixture where appropriate. "**Diastereoisomers**" are stereoisomers that have at least two asymmetric atoms, but which are not mirror-images of each other. The absolute stereochemistry is specified according to the Cahn-Ingold-Prelog R-S system. When a compound is a pure enantiomer the stereochemistry at each chiral carbon can be specified by either R or S. Resolved compounds whose absolute configuration is unknown can be designated (+) or (-) depending on the direction (dextro- or levorotatory) which they rotate plane polarized light at the wavelength of the sodium D line. Certain of the compounds described herein contain one or more asymmetric centers and can thus give rise to enantiomers, diastereomers, and other stereoisomeric forms that can be defined, in terms of absolute stereochemistry, as (R)- or (S)-. The present invention is meant to include all such possible isomers, including racemic mixtures, optically pure forms and intermediate mixtures. Optically active (R)- and (S)-isomers can be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers. Likewise, all tautomeric forms are also intended to be included.

[0031] Methods for the determination of stereochemistry and the separation of stereoisomers are well known to a person of ordinary skill in the art (see the discussion in Chapter 4 of J. March, "Advanced Organic Chemistry", 4th ed., John Wiley and Sons, New York, N.Y., 1992). When desired, the R- and S-isomers can be resolved by methods known to those skilled in the art, for example by formation of diastereoisomeric salts or complexes which can be separated, for example, by crystallisation; via formation of diastereoisomeric derivatives which can be separated, for example, by crystallisation, gas-liquid or liquid chromatography; selective reaction of one enantiomer with an enantiomer-specific reagent, for example enzymatic oxidation or reduction, followed by separation of the modified and unmodified enantiomers; or gas-liquid or liquid chromatography in a chiral environment, for example on a chiral support, such as silica with a bound chiral ligand or in the presence of a chiral solvent. It will be appreciated that where the desired enantiomer is converted into another chemical entity by one of the separation procedures described above, a further step may be required to liberate the

desired enantiomeric form. Alternatively, specific enantiomers can be synthesized by asymmetric synthesis using optically active reagents, substrates, catalysts or solvents, or by converting one enantiomer to the other by asymmetric transformation.

[0032] **Optional** or **optionally** means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, "optionally substituted alkyl" means either "alkyl" or "substituted alkyl" as defined herein. It will be understood by those skilled in the art with respect to any group containing one or more substituents that such groups are not intended to introduce any substitution or substitution patterns that are sterically impractical and/or synthetically non-feasible and/or inherently unstable.

[0033] **Pharmaceutically acceptable carrier** or **pharmaceutically acceptable excipient** includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

[0034] **Substituted-** alkyl, aryl, heteroaryl and heterocyclyl refer respectively to alkyl, aryl, heteroaryl and heterocyclyl wherein one or more (up to about 5, preferably up to about 3) hydrogen atoms are replaced by a substituent independently selected from the group: optionally substituted alkyl (e.g., fluoroalkyl), optionally substituted alkoxy, alkylenedioxy (e.g. methylenedioxy), optionally substituted amino (e.g., alkylamino and dialkylamino), optionally substituted amidino, optionally substituted aryl (e.g., phenyl), optionally substituted aralkyl (e.g., benzyl), optionally substituted aryloxy (e.g., phenoxy), optionally substituted aralkoxy (e.g., benzyloxy), carboxy (-COOH), carboalkoxy (i.e., acyloxy or -OOCR), carboxyalkyl (i.e., esters or -COOR), carboxamido, aminocarbonyl, benzyloxycarbonylamino (CBZ-amino), cyano, carbonyl, halogen, hydroxy, optionally substituted heteroaryl, optionally substituted heteroaralkyl, optionally substituted heteroaryloxy, optionally substituted heteroaralkoxy, nitro, sulfanyl, sulfinyl, sulfonyl, and thio.

[0035] **Substituted alkoxy** refers to the group -O-(substituted alkyl). One preferred substituted alkoxy group is "polyalkoxy" or -O-(optionally substituted

alkylene)-(optionally substituted alkoxy), and includes groups such as $-OCH_2CH_2OCH_3$, and glycol ethers such as polyethyleneglycol and $-O(CH_2CH_2O)_xCH_3$, where x is an integer of about 2-20, preferably about 2-10, and more preferably about 2-5. Another preferred substituted alkoxy group is hydroxyalkoxy or $-OCH_2(CH_2)_yOH$, where y is an integer of about 1-10, preferably about 1-4.

[0036] **Pharmaceutically acceptable acid addition salt** refers to those salts that retain the biological effectiveness of the free bases and that are not biologically or otherwise undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

[0037] **Pharmaceutically acceptable base addition salts** include those derived from inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Particularly preferred are the ammonium, potassium, sodium, calcium, and magnesium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine.

[0038] **Pharmaceutically acceptable esters** refers to esters of compounds of the present invention which hydrolyze in vivo and include those that break down readily in the human body to leave the parent compound or a salt thereof. Examples of pharmaceutically acceptable, non-toxic esters of the present invention include C_1 to C_6 alkyl esters and C_5 to C_7 cycloalkyl esters, although C_1 - to C_4 alkyl esters are preferred. Esters of the compounds of Formula I can be prepared according to conventional methods. Pharmaceutically acceptable, non-toxic esters of the present invention also include prodrug ester groups, i.e., any of several ester-forming groups that are hydrolyzed under physiological conditions. Examples of prodrug ester groups include pivoyloxymethyl, acetoxymethyl, phthalidyl, indanyl and methoxymethyl, as well as other such groups known in the art. Other examples of prodrug ester groups can be found

in the book "Pro-drugs as Novel Delivery Systems," by Higuchi and Stella., V. 14 of the A.C.S. Symposium Series.

[0039] **Pharmaceutically acceptable amide** refers to non-toxic amides of the present invention derived from ammonia, primary C_1 to C_6 alkyl amines and secondary C_1 to C_6 dialkyl amines. In the case of secondary amines, the amine can also be in the form of a 5- or 6-membered heterocycle containing one nitrogen atom. Amides derived from ammonia, C_1 to C_3 alkyl primary amides and C_1 to C_3 dialkyl secondary amides are preferred. Amides of the compounds of Formula I can be prepared according to conventional methods.

[0040] **Subject** or **patient** refers to an animal, preferably a mammal, that has been the object of treatment, observation or experiment, and most preferably refers to a human who is or has been the object of treatment and/or observation.

[0041] **Sulfanyl** refers to the groups: -S-(optionally substituted alkyl), -S-(optionally substituted aryl), -S-(optionally substituted heteroaryl), and -S-(optionally substituted heterocyclyl).

[0042] **Sulfinyl** refers to the groups: -S(O)-H, -S(O)-(optionally substituted alkyl), -S(O)-optionally substituted aryl), -S(O)-(optionally substituted heteroaryl), and -S(O)-(optionally substituted heterocyclyl).

[0043] **Sulfonyl** refers to the groups: -S(O_2)-H, -S(O_2)-(optionally substituted alkyl), -S(O_2)-optionally substituted aryl), -S(O_2)-optionally substituted aralkyl), -S(O_2)-(optionally substituted heteroaryl), -S(O_2)-(optionally substituted heteroaralkyl), -S(O_2)-(optionally substituted heterocyclyl) ,-S(O_2)-(optionally substituted alkoxy), -S(O_2)-optionally substituted aryloxy), -S(O_2)-(optionally substituted heteroaryloxy), and -S(O_2)-(optionally substituted heterocyclyloxy).

[0044] **Therapeutically effective amount** refers to that amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue system, animal or human that is being sought by a research, veterinarian, medical doctor or other clinician, which includes alleviation of the symptoms of the disease or disorder being treated.

[0045] **Treatment** or **treating** refers to any treatment of a disease in a subject, including:

a) preventing the disease, that is, causing the clinical symptoms of the disease not to develop;

b) inhibiting the disease, that is, slowing or arresting the development of clinical symptoms; and/or

c) relieving the disease, that is, causing the regression of clinical symptoms.

[0046] It should be understood that compounds of the invention can exist in various equilibrium forms, depending on conditions including choice of solvent, pH, and others known to the practitioner skilled in the art. All such forms of these compounds are expressly included in the present invention.

[0047] Some of the crystalline forms for the compounds can exist as polymorphs and as such are included in the present invention. In addition, some of the compounds can form solvates with water (i.e., hydrates) or common organic solvents, and such solvates are also encompassed within the scope of this invention.

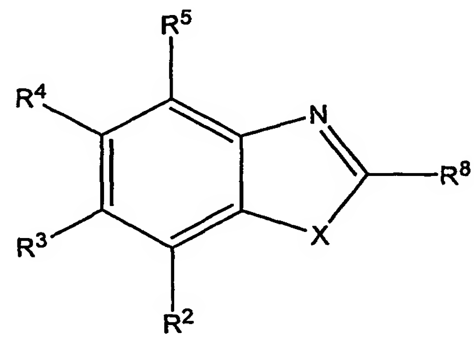
[0048] The present invention includes within its scope prodrugs of the compounds shown herein. In general, such prodrugs will be functional derivatives of the compounds that are readily convertible *in vivo* into the required compound. Thus, in the methods of treatment of the present invention, the term "administering" shall encompass the treatment of the various disorders described with the compounds specifically disclosed or with a compound which may not be specifically disclosed, but which converts to the specified compound *in vivo* after administration to a subject in need thereof. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in "Design of Prodrugs", H. Bundgaard ed., Elsevier, 1985. Protected forms of the inventive compounds are included within the scope of the present invention.

[0049] Implicit hydrogen atoms are omitted from the formulae for clarity, but should be understood to be present.

[0050] Reference will now be made in detail to certain embodiments of the invention. While the invention will be described in conjunction with certain embodiments, it should be understood that such embodiments are not intended to limit the invention to these embodiments. On the contrary, the invention is intended to cover alternatives, modifications and equivalents which are included within the spirit and scope of the invention.

Compounds of the Invention

[0051] Certain embodiments of the invention are directed to a class of novel compounds that are inhibitors of the fungal kinesin, Candida albicans Kip1. Certain embodiments of the invention also provide methods for treating fungal infection by inhibiting the fungal kinesin Kip1. The methods employ compounds represented by Formula I



Formula I

wherein

X is $-NR^1-$, $-CH=N-$, $-N=CH-$, $-CH=CH-$, S, -(SO)-, -(SO_2)-, or O;

R^1 is hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, or optionally substituted heteroaralkyl;

R^2, R^3, R^4, and R^5 are independently hydrogen, optionally substituted acyl, optionally substituted alkyl, optionally substituted aralkyl, optionally substituted heteroaralkyl, optionally substituted alkoxy, optionally substituted aralkoxy, optionally substituted heteroaralkoxy, halogen, hydroxyl, nitro, cyano, optionally substituted amino, sulfonyl, sulfanyl, carboxy, optionally substituted alkoxycarbonyl, optionally substituted aminocarbonyl, optionally substituted aryl or optionally substituted heteroaryl; or R^3 and R^4, together with the carbons to which they are attached, form an optionally substituted 5- or 6-membered alicyclic ring; and

R^8 is hydrogen, cyano, halogen, optionally substituted acyl, optionally substituted lower alkyl, optionally substituted aryl, optionally substituted heteroaryl, sulfonyl, or optionally substituted amino;

provided that

R^4 is not trifluoromethyl when X is NR^1; R^5, R^3, R^2, and R^1 are hydrogen; and R^8 is optionally substituted lower alkyl;

R^3 is not trifluoromethoxy, optionally substituted lower alkyl or halo

when X is S; R^5, R^4, and R^2 are hydrogen; and R^8 is amino;

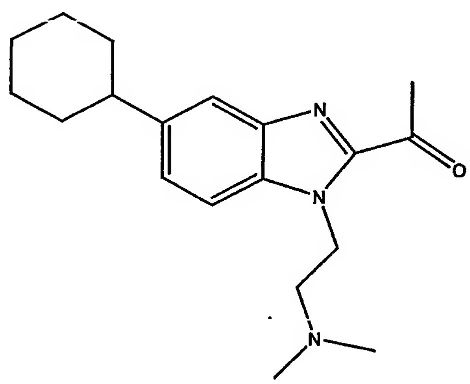
X is not S, when R^3 and R^4, together with the carbons to which they are attached form a cyclohexyl ring; R^8 is amino; and R^5 and R^2 are hydrogen; and

R^4 is not lower alkyl or halo, when X is NR^1; R^5, R^3, R^2, and R^1 are hydrogen; and R^8 is amino;

including single stereoisomers, mixtures of stereoisomers, and the pharmaceutically acceptable salts thereof.

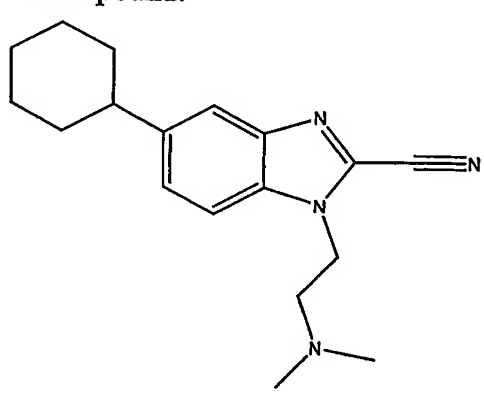
Nomenclature

[0052] The compounds of Formula I are named and numbered as described below. For example, the compound:



i.e, the compound of Formula I, wherein X is NR^1; R^1 is 2-dimethylamino-ethyl; R^2 is hydrogen; R^3 is hydrogen; R^4 is cyclohexyl; R^5 is hydrogen; and R^8 is acetyl can be named 1-[5-cyclohexyl-1-(2-dimethylamino-ethyl)-1H-benzoimidazol-2-yl]-ethanone.

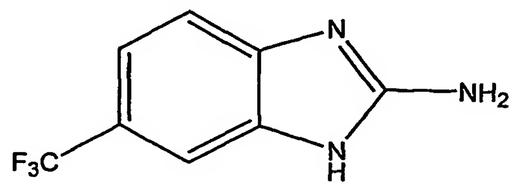
[0053] Likewise, the compound:



i.e, the compound of Formula I, wherein X is NR^1; R^1 is 2-dimethylamino-ethyl; R^2 is

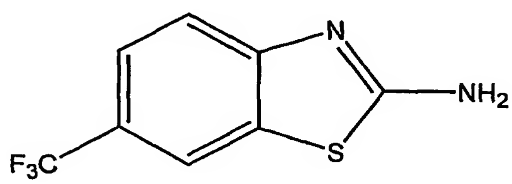
hydrogen; R^3 is hydrogen; R^4 is cyclohexyl; R^5 is hydrogen; and R^8 is cyano can be named 5-cyclohexyl-1-(2-dimethylamino-ethyl)-1H-benzoimidazole-2-carbonitrile.

[0054] Likewise, the compound:



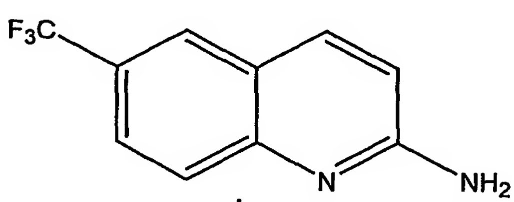
i.e., the compound of Formula I, wherein X is NR^1; R^1 is hydrogen; R^2 is hydrogen; R^3 is trifluoromethyl-; R^4 is hydrogen; R^5 is hydrogen; and R^8 is amino can be named 6-trifluoromethyl-1H-benzoimidazol-2-ylamine.

[0055] Likewise, the compound:



i.e, the compound of Formula I, wherein X is S; R^2 is hydrogen; R^3 is trifluoromethyl-; R^4 is hydrogen; R^5 is hydrogen; and R^8 is amino can be named 6-trifluoromethyl-benzothiazol-2-ylamine.

[0056] Likewise, the compound:



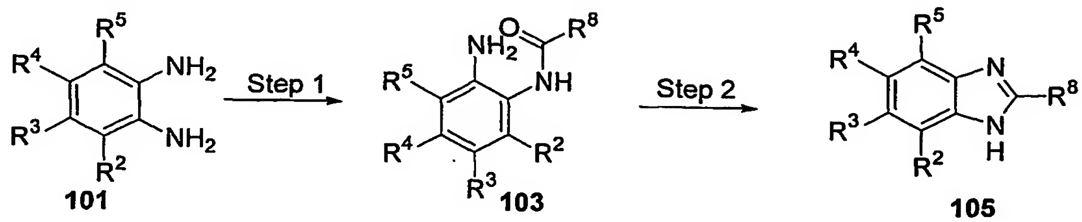
i.e, the compound of Formula I, wherein X is –CH=CH-; R^2 is hydrogen; R^3 is trifluoromethyl-; R^4 is hydrogen; R^5 is hydrogen; and R^8 is amino can be named 6-trifluoromethyl-quinolin-2-ylamine.

Synthesis of the Compounds of Formula I

[0057] The compounds of Formula I can be readily prepared by those skilled in the art using commonly employed synthetic methodology from substituted indole

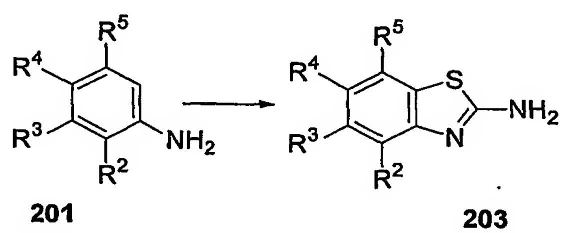
carboxylic acids that are commercially available, e.g., from Aldrich Chemical Company, Milwaukee, WI.

Reaction Scheme 1



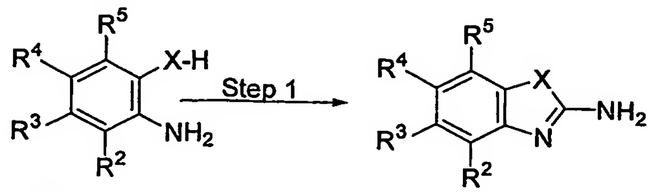
[0058] A compound of Formula R^8COCl wherein R^8 is not optionally substituted amino is added to a solution of a compound of Formula 101 and a base such as pyridine in an inert solvent such as dichloromethane. The solution is stirred at room temperature overnight and then the volatiles are removed *in vacuo* to give a solid. An acid such as toluenesulfonic acid monohydrate in an inert solvent such as xylenes is added to the crude residue and the resulting mixture is stirred at about 140°C for about 6 hours. The product, a compound of Formula 105, is isolated and optionally purified.

Reaction Scheme 2



[0059] Compounds of Formula I, wherein X is S and R^8 is optionally substituted amino can be prepared by treatment of a phenylamine of Formula 201 with an excess of bromine and either NaSCN or KSCN in acetic acid. Compounds of Formula 203 are isolated and purified.

Reaction Scheme 3

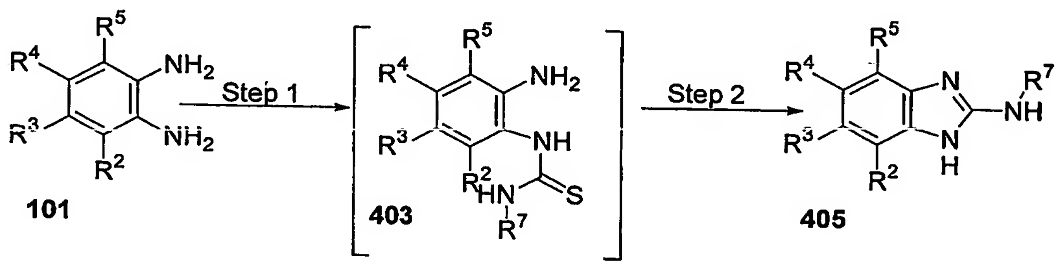


301 303

[0060] A solution of a compound of Formula 301 wherein X is S or NH and an excess of cyanogen bromide in a polar, protic solvent such as aqueous methanol is maintained at room temperature for about 8 hours. The product, a compound of Formula 303 is isolated and optionally purified.

[0061] If desired, compounds of Formula 303 can be further derivatized using techniques known in the art. For example, if any of R^2, R^3, R^4, or R^5 is a halogen, the corresponding compound wherein the halogen is optionally substituted alkyl, optionally substituted aryl, or optionally substituted heteroaryl can be prepared via a palladium-catalyzed coupling reaction.

Reaction Scheme 4



101 403 405

[0062] A compound of Formula 101 and an excess of a compound of Formula R^7HNCS (wherein R^7 is optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, or optionally substituted heteroaralkyl) in an inert solvent such as ethyl acetate are heated at about 75°C with stirring for about 2 hours. The solvent is removed and the residue is dissolved in a polar protic solvent such as ethanol. Mercury(II) oxide and sulfur are added and the mixture is heated at about 75°C for about 2 hours. The product, a compound of Formula 405, is isolated and optionally purified.

Processes and Steps

[0063] A racemic mixture of isomers of a compound of Formula I is placed on a chromatography column and separated into (R)- and (S)- enantiomers.

[0064] A compound of Formula I is contacted with a pharmaceutically acceptable acid to form the corresponding acid addition salt.

[0065] A pharmaceutically acceptable acid addition salt of Formula I is contacted with a base to form the corresponding free base of Formula I.

Compounds

[0066] When considering the compounds of the invention, X is –NR^1–, –CH=N–, –N=CH–, –CH=CH–, S, -(SO)-, -(SO_2)-, or O.

[0067] In one embodiment, X is –S-.

[0068] In another embodiment X is –NR^1– and R^1 is hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted aralkyl, or optionally substituted heteroaralkyl. In a more particular embodiment, R^1 is hydrogen.

[0069] In another embodiment, X is –NR^1– and R^1 is –(C R^{10}R^{11})$_n$-NR^{12}R^{13} wherein R^{10} and R^{11} are independently hydrogen or optionally substituted lower alkyl; n is 1, 2, or 3; and R^{12} and R^{13} are independently hydrogen, lower alkyl, or acyl; or R^{12} and R^{13} together with the nitrogen to which they are attached form an optionally substituted heterocyclyl group.

[0070] In another embodiment, X is –NR^1– and R^1 together with R^8, and the atoms to which they are attached, form an optionally substituted 5- to 7-membered heterocyclic ring. In a certain embodiment, R^8 is a substituted amino group and the position alpha to the amine is an oxo whereby R^1 together with R^8, and the atoms to which they are attached form an optionally substituted 5- to 7-membered lactam.

[0071] When considering the compounds of the invention, R^2, R^3, R^4, and R^5 are independently hydrogen, optionally substituted acyl, optionally substituted alkyl, optionally substituted aralkyl, optionally substituted heteroaralkyl, optionally substituted alkoxy, optionally substituted aralkoxy, optionally substituted heteroaralkoxy, halogen, hydroxyl, nitro, cyano, optionally substituted amino, sulfonyl, sulfanyl, carboxy, optionally substituted alkoxycarbonyl, optionally substituted aminocarbonyl, optionally substituted aryl or optionally substituted heteroaryl.

[0072] In a certain embodiment, R^2, R^3, R^4, and R^5 are independently hydrogen; hydroxyl; halogen (particularly chloro, bromo, or fluoro); optionally substituted alkyl (particularly cyclohexyl, methyl, or trifluoromethyl); optionally substituted lower alkoxy

(particularly methoxy or trifluoromethoxy); phenyl; phenyl substituted with one or more of the following substituents: halo, optionally substituted lower alkyl (particularly methyl or trifluoromethyl), and optionally substituted lower alkoxy (particularly methoxy or trifluoromethoxy); acetyl; optionally substituted aralkoxy (particularly benzyloxy); nitro; or cyano.

[0073] In a certain embodiment, only one of R^2, R^3, R^4, and R^5 is not hydrogen, especially R^3 or R^4. In another embodiment, R^4 is trifluoromethyl- and R^2, R^3, and R^5 are hydrogen. In another certain embodiment, only two of R^2, R^3, R^4, and R^5 are not hydrogen. In one embodiment, R^3 and R^4 are not hydrogen.

[0074] In a certain embodiment, R^3 and R^4, together with the carbons to which they are attached, form an optionally substituted 5- or 6-membered alicyclic ring and R^2 and R^5 are as defined above.

[0075] When considering the compounds of the invention, in one embodiment, R^8 is hydrogen, cyano, halogen, optionally substituted acyl; optionally substituted lower alkyl; optionally substituted aryl; optionally substituted heteroaryl; sulfonyl, or optionally substituted amino. In a certain embodiment, R^8 is hydrogen; amino; amino substituted with optionally substituted lower alkyl; amino substituted with sulfonyl; acyl (especially, formyl or acetyl); phenyl; phenyl substituted with lower alkyl, halo, or lower alkoxy; halogen (especially, chloro); optionally substituted lower alkyl (especially, methyl, cyclopropyl, trifluoromethyl, hydroxymethyl-, 1-hydroxyethyl-, 2-hydroxyethyl-, or 3-hydroxyprop-1yl); furan-2-yl; furan-3-yl; pyridin-2-yl; pyridin-3-yl; pyridin-4-yl; sulfonyl; or cyano.

[0076] When considering the compounds of the invention, in a particular embodiment,

X is $-NR^1-$;

R^1 is hydrogen;

R^2, R^3, R^4, and R^5 are independently hydrogen; hydroxyl; halogen; optionally substituted alkyl; acetyl; optionally substituted lower alkoxy; phenyl; phenyl substituted with one or more of the following substituents: halo, optionally substituted lower alkyl, and optionally substituted lower alkoxy; optionally substituted aralkoxy; nitro; or cyano; and

R^8 is hydrogen; amino; amino substituted with optionally substituted lower alkyl; amino substituted with sulfonyl; acyl (especially, formyl or acetyl); phenyl; phenyl

substituted with lower alkyl, halo, or lower alkoxy; halogen (especially, chloro); optionally substituted lower alkyl (especially, methyl, cyclopropyl, trifluoromethyl, hydroxymethyl-, 1-hydroxyethyl-, 2-hydroxyethyl-, or 3-hydroxyprop-1yl); furan-2-yl; furan-3-yl; pyridin-2-yl; pyridin-3-yl; pyridin-4-yl; sulfonyl; or cyano. More particularly, R^4 is trifluoromethyl-; and R^2, R^3, and R^5 are hydrogen.

[0077] When considering the compounds of the invention, in a particular embodiment,

X is S;

R^2, R^3, R^4, and R^5 are independently hydrogen; hydroxyl; halogen; optionally substituted alkyl; acetyl; optionally substituted lower alkoxy; phenyl; phenyl substituted with one or more of the following substituents: halo, optionally substituted lower alkyl, and optionally substituted lower alkoxy; optionally substituted aralkoxy; nitro; or cyano; and

R^8 is hydrogen; amino; amino substituted with optionally substituted lower alkyl; amino substituted with sulfonyl; acyl (especially, formyl or acetyl); phenyl; phenyl substituted with lower alkyl, halo, or lower alkoxy; halogen (especially, chloro); optionally substituted lower alkyl (especially, methyl, cyclopropyl, trifluoromethyl, hydroxymethyl-, 1-hydroxyethyl-, 2-hydroxyethyl-, or 3-hydroxyprop-1yl); furan-2-yl; furan-3-yl; pyridin-2-yl; pyridin-3-yl; pyridin-4-yl; sulfonyl; or cyano. More particularly, R^4 is trifluoromethyl-; and R^2, R^3, and R^5 are hydrogen.

[0078] Particular compounds for use in the methods of the invention include the following:

1-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-ethanone;

2-(4-methoxy-phenyl)-5-trifluoromethyl-1H-benzoimidazole;

(5-trifluoromethyl-1H-benzoimidazol-2-yl)-methanol;

5,6,7,8-Tetrahydro-naphtho[2,3-d]thiazol-2-ylamine

2-furan-2-yl-5-trifluoromethyl-1H-benzoimidazole;

1-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-ethanol;

2-pyridin-4-yl-5-trifluoromethyl-1H-benzoimidazole;

2-pyridin-3-yl-5-trifluoromethyl-1H-benzoimidazole;

2-cyclopropyl-5-trifluoromethyl-1H-benzoimidazole;

2-chloro-5-trifluoromethyl-1H-benzoimidazole;

5-trifluoromethyl-1H-benzoimidazole;

5-Trifluoromethyl-1H-benzoimidazol-2-ylamine;

Cyclopropyl-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;

(Tetrahydro-furan-2-ylmethyl)-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;

Ethyl-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;

Methyl-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;

N-(5-Trifluoromethyl-1H-benzoimidazol-2-yl)-methanesulfonamide;

7-Bromo-5-trifluoromethyl-1H-benzoimidazol-2-ylamine;

5,6-Dichloro-1H-benzoimidazol-2-ylamine;

4-Chloro-6-trifluoromethyl-1H-benzoimidazol-2-ylamine;

6-Chloro-5-fluoro-1H-benzoimidazol-2-ylamine;

Allyl-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;

6-Chloro-5-methyl-1H-benzoimidazol-2-ylamine;

6-tert-Butyl-1H-benzoimidazol-2-ylamine;

5-Bromo-6,7-dimethyl-1H-benzoimidazol-2-ylamine;

5-Trifluoromethyl-benzothiazol-2-ylamine;

5-Chloro-7-methyl-1H-benzoimidazol-2-ylamine;

1-(2-Amino-7-methyl-1H-benzoimidazol-4-yl)-ethanone;

6-Methyl-7-nitro-1H-benzoimidazol-2-ylamine;

5-Phenyl-1H-benzoimidazol-2-ylamine;

5-(3-Chloro-phenyl)-1H-benzoimidazol-2-ylamine;

5-(2-Chloro-phenyl)-1H-benzoimidazol-2-ylamine;

5-Isopropyl-1H-benzoimidazol-2-ylamine;

6-Bromo-5,7-dimethyl-1H-benzoimidazol-2-ylamine;

6-Benzyloxy-1H-benzoimidazol-2-ylamine;

(4-Bromo-2-methyl-phenyl)-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;

5-Bromo-1H-benzoimidazol-2-ylamine;

5-(4-Chloro-phenyl)-1H-benzoimidazol-2-ylamine;

5-o-Tolyl-1H-benzoimidazol-2-ylamine;

5-m-Tolyl-1H-benzoimidazol-2-ylamine;

5-(3-Methoxy-phenyl)-1H-benzoimidazol-2-ylamine;

5-(2-Fluoro-phenyl)-1H-benzoimidazol-2-ylamine;

5-(3-Fluoro-phenyl)-1H-benzoimidazol-2-ylamine;

5-(3-Trifluoromethoxy-phenyl)-1H-benzoimidazol-2-ylamine;

6-Chloro-5-trifluoromethyl-1H-benzoimidazol-2-ylamine;
4,6-Dimethyl-1H-benzoimidazol-2-ylamine;
5,6-Bis-trifluoromethyl-1H-benzoimidazol-2-ylamine;
5-Trifluoromethoxy-1H-benzoimidazol-2-ylamine;
6-(2-Chloro-phenyl)-benzothiazol-2-ylamine;
Methyl-(6-trifluoromethyl-benzothiazol-2-yl)-amine;
4-Chloro-6-trifluoromethyl-benzothiazol-2-ylamine;
4-Methyl-6-trifluoromethyl-benzothiazol-2-ylamine;
5-Phenyl-6-trifluoromethyl-benzothiazol-2-ylamine;
7-Phenyl-6-trifluoromethyl-benzothiazol-2-ylamine;
4,6-Bis-trifluoromethyl-benzothiazol-2-ylamine;
N-(6-Trifluoromethyl-benzothiazol-2-yl)-formamide;
5-Fluoro-6-trifluoromethyl-benzothiazol-2-ylamine;
N-(5-Trifluoromethyl-1H-benzoimidazol-2-yl)-formamide;
2-Amino-6-trifluoromethyl-benzothiazol-4-ol;
5-Methoxy-6-trifluoromethyl-benzothiazol-2-ylamine;
2-Amino-6-trifluoromethyl-benzothiazole-5-carbonitrile;
2-Amino-6-trifluoromethyl-benzothiazole-7-carbonitrile;
7-Bromo-5-trifluoromethyl-1H-benzoimidazol-2-ylamine;
6-Bromo-5,7-dimethyl-1H-benzoimidazol-2-ylamine;
5-Bromo-6,7-dimethyl-1H-benzoimidazol-2-ylamine;
4-Acetyl-7-methyl-1H-benzoimidazol-2-ylamine;
5-Chloro-7-methyl-1H-benzoimidazol-2-ylamine;
6-Ethyl-1H-benzoimidazol-2-ylamine;
6-Isopropyl-1H-benzoimidazol-2-ylamine;
6-Benzyloxy-1H-benzoimidazol-2-ylamine;
6-Tert-butyl-1H-benzoimidazol-2-ylamine;
5,6-Bis-trifluoromethyl-1H-benzoimidazol-2-ylamine;
5-Chloro-6-trifluoromethyl-1H-benzoimidazol-2-ylamine;
4-Bromo-6-trifluoromethyl-1H-benzoimidazol-2-ylamine;
4-Chloro-6-trifluoromethyl-1H-benzoimidazol-2-ylamine;
6-Trifluoromethyl-1H-benzoimidazol-2-ylamine;
4,6-Dimethyl-1H-benzoimidazol-2-ylamine;

5,6-Dimethyl-1H-benzoimidazol-2-ylamine;
6-Chloro-5-trifluoromethyl-1H-benzoimidazol-2-ylamine;
6-Chloro-5-methyl-1H-benzoimidazol-2-ylamine;
5,6-Dichloro-1H-benzoimidazol-2-ylamine;
5-Fluoro-6-chloro-1H-benzoimidazol-2-ylamine;
5-Propyl-1H-benzoimidazol-2-ylamine;
5-Trifluoromethyl-1H-benzoimidazol-2-ylamine;
5-Trifluoromethoxy-1H-benzoimidazol-2-ylamine;
5-o-Chlorophenyl-1H-benzoimidazol-2-ylamine;
5-m-Chlorophenyl-1H-benzoimidazol-2-ylamine;
5-p-Chlorophenyl-1H-benzoimidazol-2-ylamine;
5-o-Fluorophenyl-1H-benzoimidazol-2-ylamine;
5-m-Fluorophenyl-1H-benzoimidazol-2-ylamine;
5-p-Fluorophenyl-1H-benzoimidazol-2-ylamine;
5-o-Trifluoromethoxyphenyl-1H-benzoimidazol-2-ylamine;
5-m-Trifluoromethoxyphenyl-1H-benzoimidazol-2-ylamine;
5-p-Trifluoromethoxyphenyl-1H-benzoimidazol-2-ylamine;
Methyl-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;
(Tetrahydro-furan-2-ylmethyl)-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;
(Tetrahydro-furan-3-ylmethyl)-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;
(Bromomethylphenyl) -(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;
N-(5-Trifluoromethyl-1H-benzoimidazol-2-yl)-methanesulfonamide;
5-o-tolyl-1H-benzoimidazol-2-ylamine;
5-m-tolyl-1H-benzoimidazol-2-ylamine;
5-p-tolyl-1H-benzoimidazol-2-ylamine;
5-Phenyl-1H-benzoimidazol-2-ylamine;
Ethyl-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;
Allyl-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;
Cyclopropyl-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;
5-o-Methoxyphenyl-1H-benzoimidazol-2-ylamine;
5-m-Methoxyphenyl-1H-benzoimidazol-2-ylamine;
5-p-Methoxyphenyl-1H-benzoimidazol-2-ylamine;
5-o-Bromophenyl-1H-benzoimidazol-2-ylamine;

5-m-Bromophenyl-1H-benzoimidazol-2-ylamine;
5-p-Bromophenyl-1H-benzoimidazol-2-ylamine;
6-Methyl-7-nitro-1H-benzoimidazol-2-ylamine;
6-Trifluoromethyl-quinolin-2-ylamine;
6-Isopropyl-quinolin-2-ylamine;
6-tert-Butyl-quinolin-2-ylamine;
6-Trifluoromethyl-quinoxalin-2-ylamine;
6-tert-Butyl-quinoxalin-2-ylamine;
6-Trifluoromethyl-quinazolin-2-ylamine;
6-tert-Butyl-quinazolin-2-ylamine;
5-Methoxy-6-trifluoromethyl-benzothiazol-2-ylamine;
4-Methyl-6-trifluoromethyl-benzothiazol-2-ylamine;
4-Hydroxy-6-trifluoromethyl-benzothiazol-2-ylamine;
5-Cyano-6-trifluoromethyl-benzothiazol-2-ylamine;
5-Fluoro-6-trifluoromethyl-benzothiazol-2-ylamine;
5-Phenyl-6-trifluoromethyl-benzothiazol-2-ylamine;
3-Phenyl-6-trifluoromethyl-benzothiazol-2-ylamine;
3-Cyano-6-trifluoromethyl-benzothiazol-2-ylamine;
5-trifluoromethyl-benzothiazol-2-ylamine;
6-Trifluoromethoxy-benzothiazol-2-ylamine;
3-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-propan-1-ol;
6-Trifluoromethyl-benzothiazol-2-ylamine;
6-bromo-benzothiazol-2-ylamine;
6-isopropyl-benzothiazol-2-ylamine;
6-tert-Butyl-benzothiazol-2-ylamine;
5-Ethyl-1H-benzoimidazol-2-ylamine;
5-Chloro-1H-benzoimidazol-2-ylamine;
2,5-bis-trifluoromethyl-1H-benzoimidazole; and
2-methyl-5-trifluoromethyl-1H-benzoimidazole.

[0079] Particular compounds of the invention include
1-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-ethanone;
2-(4-methoxy-phenyl)-5-trifluoromethyl-1H-benzoimidazole;
(5-trifluoromethyl-1H-benzoimidazol-2-yl)-methanol;

2-furan-2-yl-5-trifluoromethyl-1H-benzoimidazole;

1-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-ethanol;

2-pyridin-4-yl-5-trifluoromethyl-1H-benzoimidazole;

2-pyridin-3-yl-5-trifluoromethyl-1H-benzoimidazole;

2-cyclopropyl-5-trifluoromethyl-1H-benzoimidazole;

2-chloro-5-trifluoromethyl-1H-benzoimidazole;

5-trifluoromethyl-1H-benzoimidazole;

5-Trifluoromethyl-1H-benzoimidazol-2-ylamine;

Cyclopropyl-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;

(Tetrahydro-furan-2-ylmethyl)-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;

Ethyl-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;

Methyl-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;

N-(5-Trifluoromethyl-1H-benzoimidazol-2-yl)-methanesulfonamide;

7-Bromo-5-trifluoromethyl-1H-benzoimidazol-2-ylamine;

5,6-Dichloro-1H-benzoimidazol-2-ylamine;

4-Chloro-6-trifluoromethyl-1H-benzoimidazol-2-ylamine;

6-Chloro-5-fluoro-1H-benzoimidazol-2-ylamine;

Allyl-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;

6-Chloro-5-methyl-1H-benzoimidazol-2-ylamine;

6-tert-Butyl-1H-benzoimidazol-2-ylamine;

5-Bromo-6,7-dimethyl-1H-benzoimidazol-2-ylamine;

5-Trifluoromethyl-benzothiazol-2-ylamine;

5-Chloro-7-methyl-1H-benzoimidazol-2-ylamine;

1-(2-Amino-7-methyl-1H-benzoimidazol-4-yl)-ethanone;

6-Methyl-7-nitro-1H-benzoimidazol-2-ylamine;

5-Phenyl-1H-benzoimidazol-2-ylamine;

5-(3-Chloro-phenyl)-1H-benzoimidazol-2-ylamine;

5-(2-Chloro-phenyl)-1H-benzoimidazol-2-ylamine;

5-Isopropyl-1H-benzoimidazol-2-ylamine;

6-Bromo-5,7-dimethyl-1H-benzoimidazol-2-ylamine;

6-Benzyloxy-1H-benzoimidazol-2-ylamine;

(4-Bromo-2-methyl-phenyl)-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;

5-Bromo-1H-benzoimidazol-2-ylamine;

5-(4-Chloro-phenyl)-1H-benzoimidazol-2-ylamine;
5-o-Tolyl-1H-benzoimidazol-2-ylamine;
5-m-Tolyl-1H-benzoimidazol-2-ylamine;
5-(3-Methoxy-phenyl)-1H-benzoimidazol-2-ylamine;
5-(2-Fluoro-phenyl)-1H-benzoimidazol-2-ylamine;
5-(3-Fluoro-phenyl)-1H-benzoimidazol-2-ylamine;
5-(3-Trifluoromethoxy-phenyl)-1H-benzoimidazol-2-ylamine;
6-Chloro-5-trifluoromethyl-1H-benzoimidazol-2-ylamine;
4,6-Dimethyl-1H-benzoimidazol-2-ylamine;
5,6-Bis-trifluoromethyl-1H-benzoimidazol-2-ylamine;
5-Trifluoromethoxy-1H-benzoimidazol-2-ylamine;
6-(2-Chloro-phenyl)-benzothiazol-2-ylamine;
Methyl-(6-trifluoromethyl-benzothiazol-2-yl)-amine;
4-Chloro-6-trifluoromethyl-benzothiazol-2-ylamine;
4-Methyl-6-trifluoromethyl-benzothiazol-2-ylamine;
5-Phenyl-6-trifluoromethyl-benzothiazol-2-ylamine;
7-Phenyl-6-trifluoromethyl-benzothiazol-2-ylamine;
4,6-Bis-trifluoromethyl-benzothiazol-2-ylamine;
N-(6-Trifluoromethyl-benzothiazol-2-yl)-formamide;
5-Fluoro-6-trifluoromethyl-benzothiazol-2-ylamine;
N-(5-Trifluoromethyl-1H-benzoimidazol-2-yl)-formamide;
2-Amino-6-trifluoromethyl-benzothiazol-4-ol;
5-Methoxy-6-trifluoromethyl-benzothiazol-2-ylamine;
2-Amino-6-trifluoromethyl-benzothiazole-5-carbonitrile; and
2-Amino-6-trifluoromethyl-benzothiazole-7-carbonitrile.
7-Bromo-5-trifluoromethyl-1H-benzoimidazol-2-ylamine;
6-Bromo-5,7-dimethyl-1H-benzoimidazol-2-ylamine;
5-Bromo-6,7-dimethyl-1H-benzoimidazol-2-ylamine;
4-Acetyl-7-methyl-1H-benzoimidazol-2-ylamine;
5-Chloro-7-methyl-1H-benzoimidazol-2-ylamine;
6-Ethyl-1H-benzoimidazol-2-ylamine;
6-Isopropyl-1H-benzoimidazol-2-ylamine;
6-Benzyloxy-1H-benzoimidazol-2-ylamine;

6-Tert-butyl-1H-benzoimidazol-2-ylamine;

5,6-Bis-trifluoromethyl-1H-benzoimidazol-2-ylamine;

5-Chloro-6-trifluoromethyl-1H-benzoimidazol-2-ylamine;

4-Bromo-6-trifluoromethyl-1H-benzoimidazol-2-ylamine;

4-Chloro-6-trifluoromethyl-1H-benzoimidazol-2-ylamine;

6-Trifluoromethyl-1H-benzoimidazol-2-ylamine;

4,6-Dimethyl-1H-benzoimidazol-2-ylamine;

5,6-Dimethyl-1H-benzoimidazol-2-ylamine;

6-Chloro-5-trifluoromethyl-1H-benzoimidazol-2-ylamine;

6-Chloro-5-methyl-1H-benzoimidazol-2-ylamine;

5,6-Dichloro-1H-benzoimidazol-2-ylamine;

5-Fluoro-6-chloro-1H-benzoimidazol-2-ylamine;

5-Propyl-1H-benzoimidazol-2-ylamine;

5-Trifluoromethyl-1H-benzoimidazol-2-ylamine;

5-Trifluoromethoxy-1H-benzoimidazol-2-ylamine;

5-o-Chlorophenyl-1H-benzoimidazol-2-ylamine;

5-m-Chlorophenyl-1H-benzoimidazol-2-ylamine;

5-p-Chlorophenyl-1H-benzoimidazol-2-ylamine;

5-o-Fluorophenyl-1H-benzoimidazol-2-ylamine;

5-m-Fluorophenyl-1H-benzoimidazol-2-ylamine;

5-p-Fluorophenyl-1H-benzoimidazol-2-ylamine;

5-o-Trifluoromethoxyphenyl-1H-benzoimidazol-2-ylamine;

5-m-Trifluoromethoxyphenyl-1H-benzoimidazol-2-ylamine;

5-p-Trifluoromethoxyphenyl-1H-benzoimidazol-2-ylamine;

Methyl-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;

(Tetrahydro-furan-2-ylmethyl)-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;

(Tetrahydro-furan-3-ylmethyl)-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;

(Bromomethylphenyl) -(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;

N-(5-Trifluoromethyl-1H-benzoimidazol-2-yl)-methanesulfonamide;

5-o-tolyl-1H-benzoimidazol-2-ylamine;

5-m-tolyl-1H-benzoimidazol-2-ylamine;

5-p-tolyl-1H-benzoimidazol-2-ylamine;

5-Phenyl-1H-benzoimidazol-2-ylamine;

Ethyl-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;
Allyl-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;
Cyclopropyl-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;
5-o-Methoxyphenyl-1H-benzoimidazol-2-ylamine;
5-m-Methoxyphenyl-1H-benzoimidazol-2-ylamine;
5-p-Methoxyphenyl-1H-benzoimidazol-2-ylamine;
5-o-Bromophenyl-1H-benzoimidazol-2-ylamine;
5-m-Bromophenyl-1H-benzoimidazol-2-ylamine;
5-p-Bromophenyl-1H-benzoimidazol-2-ylamine;
6-Methyl-7-nitro-1H-benzoimidazol-2-ylamine;
6-Trifluoromethyl-quinolin-2-ylamine;
6-Isopropyl-quinolin-2-ylamine;
6-tert-Butyl-quinolin-2-ylamine;
6-Trifluoromethyl-quinoxalin-2-ylamine;
6-tert-Butyl-quinoxalin-2-ylamine;
6-Trifluoromethyl-quinazolin-2-ylamine;
6-tert-Butyl-quinazolin-2-ylamine;
5-Methoxy-6-trifluoromethyl-benzothiazol-2-ylamine;
4-Methyl-6-trifluoromethyl-benzothiazol-2-ylamine;
4-Hydroxy-6-trifluoromethyl-benzothiazol-2-ylamine;
5-Cyano-6-trifluoromethyl-benzothiazol-2-ylamine;
5-Fluoro-6-trifluoromethyl-benzothiazol-2-ylamine;
5-Phenyl-6-trifluoromethyl-benzothiazol-2-ylamine;
3-Phenyl-6-trifluoromethyl-benzothiazol-2-ylamine;
3-Cyano-6-trifluoromethyl-benzothiazol-2-ylamine; and
5-trifluoromethyl-benzothiazol-2-ylamine.

[0080] In certain embodiments, compounds of the invention include
6-Trifluoromethyl-benzothiazol-2-ylamine;
6-(2-Chloro-phenyl)-benzothiazol-2-ylamine;
6-Trifluoromethoxy-benzothiazol-2-ylamine;
4-Methyl-6-trifluoromethyl-benzothiazol-2-ylamine;
2-Amino-6-trifluoromethyl-benzothiazol-4-ol;
4-Chloro-6-trifluoromethyl-benzothiazol-2-ylamine;

6-Trifluoromethoxy-benzothiazol-2-ylamine;

6-tert-Butyl-benzothiazol-2-ylamine;

5-(3-Trifluoromethoxy-phenyl)-1H-benzoimidazol-2-ylamine;

5-(3-Chloro-phenyl)-1H-benzoimidazol-2-ylamine;

5-(4-Chloro-phenyl)-1H-benzoimidazol-2-ylamine;

5-(2-Chloro-phenyl)-1H-benzoimidazol-2-ylamine;

Furan-2-yl-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;

Thiophen-2-yl-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;

(4-Methoxy-phenyl)-(5-trifluoromethyl-1H-benzoimidazol-2-yl)-amine;

2-Chloro-5-trifluoromethyl-1H-benzoimidazole;

6-Isopropyl-quinolin-2-ylamine;

6-Isopropyl-7-methyl-quinolin-2-ylamine;

6-Trifluoromethyl-quinolin-2-ylamine; and

6-sec-Butyl-quinolin-2-ylamine.

Testing

[0081] Test compounds can be assayed in a highly parallel fashion by using multiwell plates and placing the compounds either individually in wells or testing the compounds in mixtures. Assay components including the target protein complex, coupling enzymes and substrates, and ATP can then be added to the wells and the absorbance or fluorescence of each well of the plate can be measured with a plate reader.

[0082] In a certain embodiment, the method uses a 384 well plate format and a 25 μL reaction volume. A pyruvate kinase/lactate dehydrogenase coupled enzyme system (Huang et al. (1994) J Biol Chem 269(23):16493-501, which is incorporated herein by reference) is used to measure the rate of ATP hydrolysis in each well. As will be appreciated by those skilled in the art, the assay components are added in buffers and reagents. Since the methods outlined herein allow kinetic measurements, the incubation periods are optimized to give adequate detection signals over the background. The assay is done in real time giving the kinetics of ATP hydrolysis which increases the signal to noise ratio of the assay.

Therapeutic Utilities

[0083] Compounds of Formula I exhibit antifungal activity. For example, the

compounds of Formula I inhibit the growth of various infectious fungi including *Candida spp.* such as *Candida albicans*, *Candida tropicalis*, *Candida (Torulopsis) glabrata*, *Candida parapsilosis*, *Candida lusitaneae*, *Candida rugosa* and *Candida pseudotropicalis*. Fungal infections which can be inhibited or treated with compositions identified using the methods provided herein include but are not limited to: candidiasis including but not limited to onchomycosis, chronic mucocutaneous candidiasis, oral candidiasis, epiglottistis, esophagitis, gastrointestinal infections, and genitourinary infections, for example, caused by any *Candida* species, including those listed above.

[0084] A variety of cell-based assays can be used to determine activity. Among these are microtiter plate, disc plate diffusion, and inhibition of fungal hyphae length. These assays utilize standard techniques that are well-known in the art ((R.N. Jones et al, Manual of Clinical Microbiology, 4th ed., (1985); and M.A. Pfaller et al., Antimicrobial Agents and Chemotherapy, 34 (1990)).

[0085] Antifungal activity of a test compound can be determined in vitro by obtaining the minimum inhibitory concentration (MIC) of the compound using a standard agar dilution test or a disc-diffusion test. The compound is then tested in vivo (in mice) to determine the effective dose of the test compound for controlling a systemic fungal infection.

[0086] Accordingly, representative compounds of the present invention are tested for, and display, antifungal activity against at least one of the following fungi: *C. albicans, C. parapsilosis, C. neoformans, Histoplasma spp,* and *A. fumigatus*. Thus, in one embodiment, the invention herein includes application to cells or individuals afflicted or impending affliction with any one of these disorders or states.

[0087] Accordingly, the compositions of the invention can be administered to cells. By "administered" herein is meant administration of a therapeutically effective amount or dose of at least one compound of the invention to a cell either in cell culture or in a subject. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques. As is known in the art, adjustments for systemic versus localized delivery, age, body weight, general health, sex, diet, time of administration, drug interaction and the severity of the condition may be necessary, and will be ascertainable with routine experimentation by those skilled in the art.

[0088] Compounds having the desired pharmacological activity can be

administered in a physiologically acceptable carrier to a subject, as described herein. Depending upon the manner of introduction, the compounds can be formulated in a variety of ways as discussed herein. The concentration of therapeutically active compound in the formulation can vary from about 0.1-100 wt.%. The agents can be administered alone or in combination with other treatments, i.e., other agents for the treatment of fungal infection.

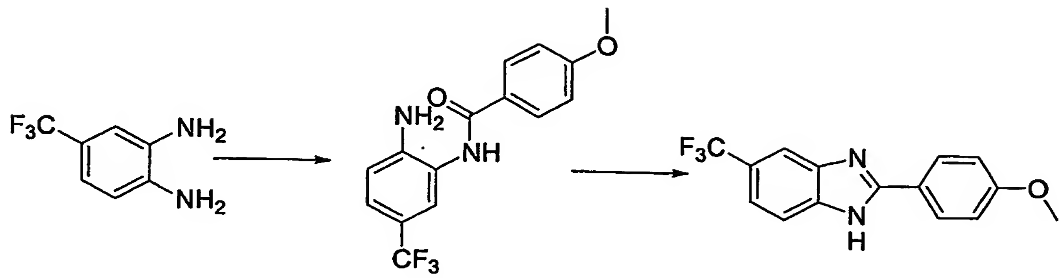
[0089] In certain embodiments, the pharmaceutical compositions are in a water soluble form, such as pharmaceutically acceptable salts, which is meant to include both acid and base addition salts. The pharmaceutical compositions can be prepared in various forms, such as granules, tablets, pills, suppositories, capsules, suspensions, salves, lotions and the like. Pharmaceutical grade organic or inorganic carriers and/or diluents suitable for oral and topical use can be used to make up compositions containing the therapeutically-active compounds. Diluents known to the art include aqueous media, vegetable and animal oils and fats. Stabilizing agents, wetting and emulsifying agents, salts for varying the osmotic pressure or buffers for securing an adequate pH value, and skin penetration enhancers can be used as auxiliary agents. The pharmaceutical compositions can also include one or more of the following: carrier proteins such as serum albumin; buffers; fillers such as microcrystalline cellulose, lactose, corn and other starches; binding agents; sweeteners and other flavoring agents; coloring agents; and polyethylene glycol. Additives are well known in the art, and can be used in a variety of formulations.

[0090] The administration of the compounds of the present invention can be done in a variety of ways as discussed herein, including, but not limited to, orally, subcutaneously, intravenously, intranasally, transdermally, intraperitoneally, intramuscularly, intrapulmonary, vaginally, rectally, or intraocularly. In some instances, for example, in the treatment of wounds and inflammation, the compounds can be directly applied as a solution or spray.

[0091] It is understood that this invention is not limited to the particular methodology, protocols, and reagents described, as these can vary. It is also understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. It is to be further understood that the above description is intended to be illustrative and not restrictive. Many embodiments will be apparent to

those skilled in the art upon reviewing the above description.

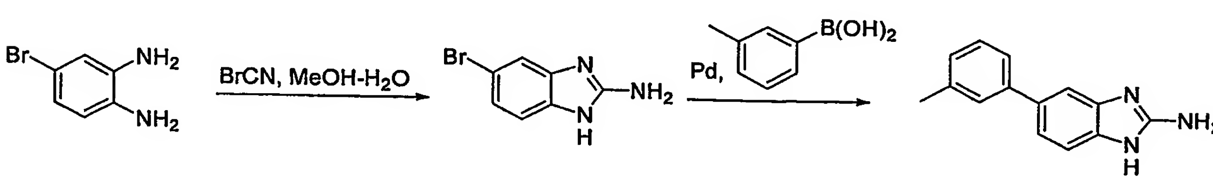
Example 1



[0092] 4-Anisoyl Chloride (110 µl, 0.75 mmol) was added to a solution of 3,4-diaminobenzotrifluoride (60 mg, 0.34 mmol), pyridine (100 µl, 0.75 mmol), and dichloromethane (900 µl, 0.3 M). The solution was stirred at room temperature overnight and then the volatiles were removed *in vacuo* to give a solid. Toluenesulfonic acid monohydrate (129 mg, 0.68 mmol) and xylene (2.5 mL) were added to the crude residue and the resulting mixture stirred at 140 °C for 6 hours. At this time the solution was allowed to cool and the less dense liquid decanted. The remaining viscous oil was diluted in ethyl acetate (4 mL) and washed with 1 N NaOH (4 mL). The mixture was passed through a filter tube which was densely packed with the hydroxy matrix (10 g) and the filtrate was concentrated *in vacuo*. The crude material was purified by HPLC and the desired product isolated as a white solid (50 mg, 55 % overall yield): MS m/z = 293.2 $(M)^+$.

Example 2

Preparation of 2-amino-5-(3'-methylphenyl)-benzimidazole:



1 2 3

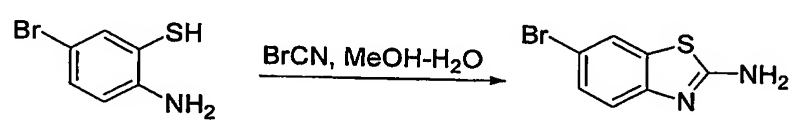
[0093] A solution of 4-bromo-2-aminoaniline (213 mg, 1.14 mmol), cyanogen bromide (358 mg, 3.42 mmol), and 50% MeOH-H2O (5.7 mL) was maintained at room

temperature for 8 hours. At this time, the solution wasconcentrated to remove MeOH, and the aqueous layer was neutralized by adding 1 M NaOH. Filtration and drying of the resulting solid gave 2-amino-5-bromo-benzimidazole (120 mg, 0.57 mmol): the compound prepared showed a molecular ion M+ = 212.0.

[0094] A mixture of 2-amino-5-bromo-benzimidazole (210 mg, 1 mmol), 3-methyl phenylboronic acid (176 mg, 1.3 mmol), $Pd(PPh_3)_4$ (115 mg, 0.1 mmol), 2 M aqueous Na_2CO_3 (1.3 mL, 2.6 mmol), and toluene (5 mL) was heated to 100 C for 12 hours. At this time, the reaction mixture was cooled and the layers were separated. The toluene layer washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated to a solid. Purification of the crude material by silica gel flash chromatography using a 0% hexanes to 60% hexanes-EtOAc gradient gave 2-amino-5-(3'-methylphenyl)-benzimidazole (178 mg, 0.8 mmol). The compound prepared showed a molecular ion M+ = 224.1.

Example 3

Preparation of 2-amino-6-bromo-benzothiazole:

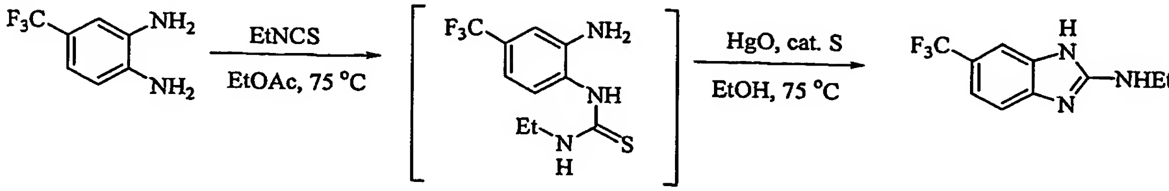


1 **2**

[0095] A solution of 2-amino-5-bromothiophenol (225 mg, 1.10 mmol), cyanogen bromide (358 mg, 3.42 mmol), and 50% MeOH-H_2O (5.7 mL) was maintained at room temperature for 8 hours. At this time, the solution was concentrated to remove MeOH, and the aqueous layer was neutralized by adding 1 M NaOH. Filtration and drying of the resulting solid gave 2-amino-6-bromo-benzothiazole (114 mg, 0.50 mmol): the compound prepared showed a molecular ion M+ = 229.0.

Example 4

General procedure for synthesis of 2-aminoethyl-6-trifluoromethyl-benzoimidazole

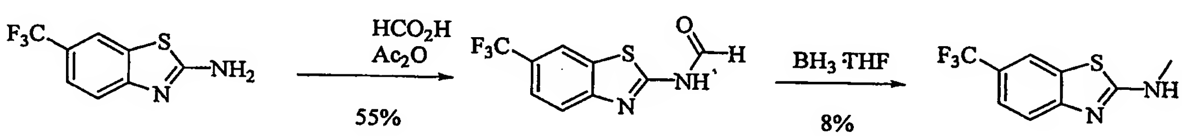


1 2 3

[0096] 4-Trifluoromethyl-benzene-1,2-diamine (1) (200 mg, 1.13 mmol), ethylisothiocyanate (147 mg, 1.69 mmol), and ethyl acetate (1.0 mL) were heated at 75 °C with stirring for 2 hours. The ethyl acetate was evaporated under a stream of nitrogen and the residue redissolved in ethanol (1.0 mL). Mercury(II) oxide (0.49 g, 2.26 mmol) and sulfur (7 mg, 0.23 mmol) were added and the mixture heated at 75 °C for 2 hours. The mixture was cooled, filtered through celite, and chromatographed on silica (2:1 dichloromethane/ethyl acetate). The compound prepared showed a molecular ion M+ = 230.1.

Example 5

Preparation of 2-Aminomethyl-5-trfluoromethyl-benzothiazole



1 2 3

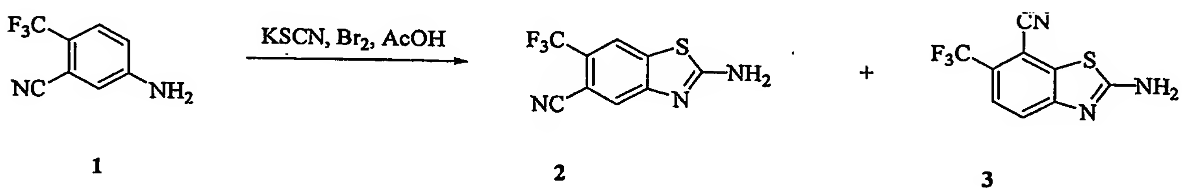
[0097] 1.66 mL (44 mmol) of formic acid was added dropwise to 3.4 mL (36 mmol) of acetic anhydride at 0 °C. The mixture was then heated to 60 °C for 2 h and then cooled to rt and diluted with 2 mL of tetrahydrofuran. 218 mg (1.0 mmol) of 6-trifluoromethyl-benzothiazol-2-ylamine (1) was then added in 4 mL of tetrahydrofuran and stirred for 2 h. The reaction was concentrated and the residue recrystallized from methanol to yield 155 mg (55%) of white solid. ^{1}H NMR (CD_3OD): 8.60 (bs, 1H), 8.25 (s, 1H), 7.87 (d, 1H), 7.7 (d, 1H).

[0098] 218 mg (1.0 mmol) of 6-trifluoromethyl-benzothiazol-2-ylamine (1) was

treated as described above to yield crude (2) after removal of solvent. The residue was dissolved in tetrahydrofuran (6 mL), cooled in an ice-bath, and 4 mL of 1M borane-tetrahydrofuran complex added. After 2 h, moist ether (5 mL) was added followed by water (1 mL) and the product extracted with ethyl acetate (2 x 20 mL). The ethyl acetate was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. Prep TLC (5% methanol/dichloromethane) yielded 12 mg (12%) of white solid. ^1H NMR ($CDCl_3$) 7.86 (s, 1H), 7.58 (m, 2H), 5.90 (bs, 1H), 3.12 (s, 3H).

Example 6

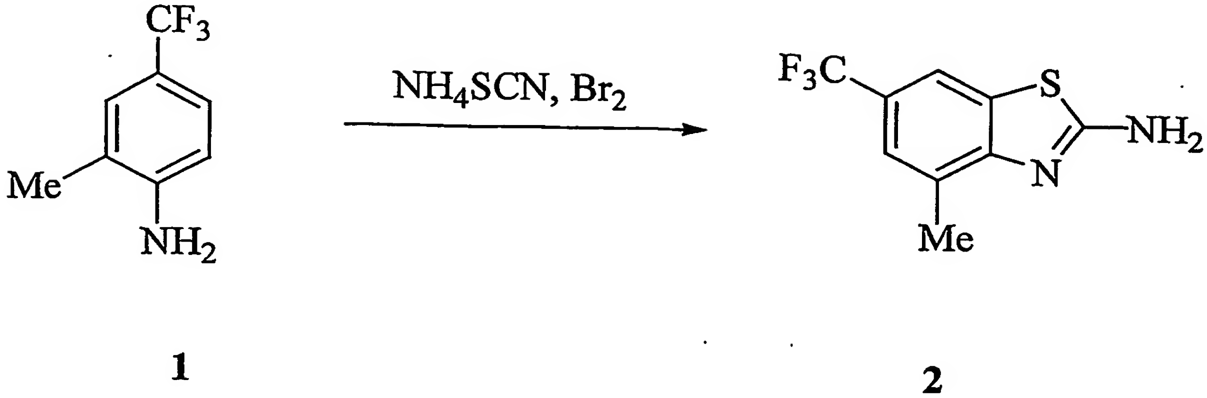
Preparation of 2-amino-5-cyano-6-trifluoromethyl-benzothiazole and 2-amino-7-cyano-6-trifluoromethylbenzothiazole



[0099] 236 mg (1.0 mmol) of 5-amino-2-trifluoromethyl-benzonitrile (1) was dissolved in a mixture of 30 mL acetic acid and 1.85 g of potassium thiocyanate (19 mmol) and cooled to 0 °C in an ice-bath. Bromine (0.45 mL, 8.8 mmol) was added dropwise and the reaction allowed to warm to rt. After 16 h, the reaction was concentrated under reduced pressure, treated with saturated $NaHCO_3$ (30 mL), and the resulting solid collected by filtration. Silica chromatography (1:1 ethyl acetate/hexanes) yielded 2-amino-5-cyano-6-trifluormethylbenzothiazole (71 mg, 29%) and 2-amino-7-cyano-6-trifluormethylbenzothiazole (61 mg, 25%) as a white solids: both compounds prepared showed a molecular ion M+ = 244.0.

Example 7

Preparation of 2-amino-4-methyl-6-trifluoromethyl-benzothiazole



[00100] 100 mg (0.56 mmol) of 2-methyl-4-trifluoromethyl-phenylamine (1) and 88 mg (1.12 mmol) of ammonium thiocyanate were combined in 2.0 mL of acetic acid and cooled to ~ 15 °C in a water bath. 120 uL of 0.3 M bromine/acetic acid solution was added dropwise over 5 minutes, resulting in a dark purple solution. The mixture was allowed to warm to room temperature and then stirred at room temperature for 2 days. The reaction mixture was then diluted with 50 mL of water and extracted with 50 mL of ethyl acetate. The ethyl acetate layer was washed once with saturated $NaHCO_3$ (50 mL), brine (50 mL), and dried with Na_2SO_4. The solution was concentrated and chromatographed on silica (20% ethyl acetate/hexanes) to give 21 mg (16%) of off-white solid. ^{1}H NMR (d_6-DMSO) 7.90 (s, 1H), 7.85 (s, 2H), 7.34 (s, 1H), 2.43 (s, 3H).

Example 8

Calculation of IC_{50}:

[0001] An ATPase assay was used to measure the IC_{50} for Kip1 activity of the compounds disclosed herein. The following solutions are used: Solution 1 consists of 3 mM phosphoenolpyruvate potassium salt (Sigma P-7127), 2 mM ATP (Sigma A-3377), 1 mM IDTT (Sigma D-9779), 5 μM paclitaxel (Sigma T-7402), 10 ppm antifoam 289 (Sigma A-8436), 25 mM Pipes/KOH pH 6.8 (Sigma P6757), 2 mM $MgCl_2$ (VWR JT400301), and 1 mM EGTA (Sigma E3889). Solution 2 consists of 1 mM NADH (Sigma N8129), 0.2 mg/mL BSA (Sigma A7906), pyruvate kinase 7U/mL, L-lactate dehydrogenase 10 U/mL (Sigma P0294), 100 nM Kip1 motor domain, 50 μg/mL microtubules, 1 mM DTT (Sigma D9779), 5 μM paclitaxel (Sigma T-7402), 10 ppm antifoam 289 (Sigma A-8436), 25 mM Pipes/KOH pH 6.8 (Sigma P6757), 2 mM $MgCl_2$ (VWR JT4003-01), and 1 mM EGTA (Sigma E3889). Serial dilutions (8-12 two-fold dilutions) of the compound are made in a 96-well microtiter plate (Corning Costar 3695)

using Solution 1. Following serial dilution each well has 50 μl of Solution 1. The reaction is started by adding 50 μl of solution 2 to each well. This may be done with a multichannel pipettor either manually or with automated liquid handling devices. The microtiter plate is then transferred to a microplate absorbance reader and multiple absorbance readings at 340 nm are taken for each well in a kinetic mode. The observed rate of change, which is proportional to the ATPase rate, is then plotted as a function of the compound concentration. For a standard IC_{50} determination the data acquired is fit by the following four parameter equation using a nonlinear fitting program (e.g., Grafit 4):

$$y = \frac{\text{Range}}{1 + \left(\frac{x}{IC_{50}}\right)^{s}} + \text{Background}$$

where y is the observed rate and x is the compound concentration.